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Simalin A and B: Two new aromatic compounds from the stem bark of *Bombax ceiba*

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ABSTRACT

Two new aromatic compounds, simalin A (**1**) and B (**2**), along with five known compounds shamiminol (**3**), (−)-epicatechin-7-*O*-*β*-xylopyranoside (**4**), (−)-catechin-7-*O*-*β*-xylopyranoside (**5**), (+)-isolarisiresinol-9′-*O*-*β*-glucopyranoside (**6**) and (+)-lyoniresinol-9′-*O*-*β*-glucopyranoside (**7**) were isolated from the stem bark of *Bombax ceiba*. Their structures were elucidated by chemical and spectral methods. Compounds **6** and **7** were isolated for the first time from *B. ceiba*.

1. Introduction

Bombax ceiba L. (Syn: *Bombax malabaricum* DC.) (family Bombacaceae) is a deciduous tree distributed throughout Nepal, India, West China and Malaysia. It is locally known as "Simal" in Nepal. Traditionally in Nepal, the bark of this plant are used in wound healing. Flowers, bark and fruits are used in revitalizing sexual impotency and gum is used as remedy of diarrhea, dysentery, influenza and menorrhagia (Gewali, 2008; Watanabe et al., 2005). In the previous studies, the methanolic extract of bark of *B. ceiba* showed *α*-glucosidase inhibitory activity (Khan et al., 2010). Earlier chemical investigations on the stem bark have led to the isolation of shamimicin, lupeol (Aleem et al., 2003), mangiferin, epicatechin-7-*O*-*β*-xylopyranoside, epicatechin-3-*O*-*β*-xylopyranoside (Khan et al., 2010), shamiminol, stigmasta-3,5-diene, lupenone, (±)-lyoniresinol-2a-*O*-*β*-D-glucopyranoside and opuntiol (Faizi et al., 2011). Our recent phytochemical studies resulted in the identification of flavonoids, xanthones, coumarin and other aromatic compounds from the flowers of this plant (Joshi et al., 2013). In the continuation, we describe herein the isolation and structure elucidation of two new (**1**–**2**) (Fig. 1) and five known (**3**–**7**) compounds from the stem bark of *B. ceiba* from Nepal.

2. Results and discussion

Successive extraction of the shade dried stem bark (1.1 kg) of *B. ceiba* with 70% MeOH, MeOH and water gave total 118 g of extract. The water soluble fraction of the extract was applied on repeated column chromatography on MCI gel CHP20P, Sephadex LH-20, ODS and silica gel to isolate the compounds **1**–**7**.

Compound **1** was obtained as colorless gum with positive optical rotation, $[\alpha]_D^{20}$ +80.4°. Its molecular formula was determined as $C_{20}H_{28}O_{14}$ on the basis of a HR-FAB-MS peak of $[M+Na]^+$ at 515.1380 (Calcd. for $C_{20}H_{28}O_{14}Na$, 515.1377). The ^1H NMR spectrum of compound **1** showed three aromatic proton signals at δ_H 7.75 (1H, d, J = 2.1 Hz), 7.58 (1H, d, J = 2.1, 7.9 Hz) and 6.85 (1H, d, J = 7.9 Hz) revealing the presence of an ABX system in a 1,3,4-trisubstituted phenyl ring. Three protons at δ_H 3.90 (3H, s) indicated the presence of one methoxy group. The proton signals at δ_H 5.12 (2H, d, J = 3.7 Hz), 4.52 (1H, dd, J = 2.1, 11.9 Hz), 4.40 (1H, dd, J = 5.8, 11.9 Hz), 3.82 (1H, ddd, J = 3.9, 5.2, 9.8 Hz), 3.66 (1H, dd, J = 5.2, 11.9 Hz), 3.53 (2H, dd, J = 3.7, 9.8 Hz) and other overlapped signals ranging from δ_H 3.40 to δ_H 4.18 indicated the presence of a disaccharide moiety. Among these, a doublet at δ_H 5.12 (2H, d, J = 3.7 Hz) was assignable to the two anomaric protons of disaccharide moiety in α,α configuration. The ^{13}C NMR spectra of compound **1** revealed the presence of 20 carbon signals including twelve signals (95.2, 95.1, 74.8, 74.6, 73.9, 73.3, 73.2, 72.2, 71.9, 71.6, 65.0 and 62.6) assignable to disaccharide moiety; one ester carbonyl carbon at δ_C 168.1 (C=O); six aromatic carbons at δ_C 152.9 (C), 148.8 (C), 125.2 (CH), 122.6 (C), 116.0 (CH), 113.7

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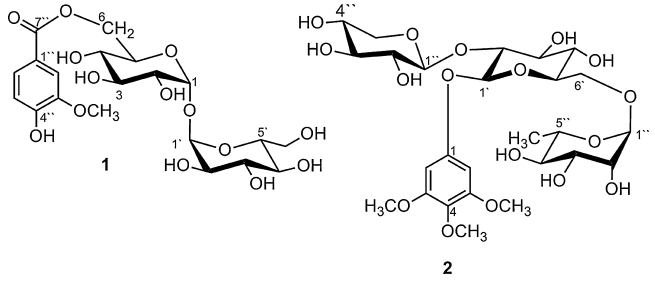

Fig. 1. Structure of compounds **1**–**2**.

(CH) and one methoxy group at δ_C 56.5 (OCH_3). These ^{13}C NMR signals of disaccharide part were almost similar with α,α-trehalose except two CH_2 group at δ_C 65.0 and 62.6 indicated the substitution at C-6 position of a one glucose moiety of α,α-trehalose (Paul et al., 2012). The disaccharide moiety was confirmed by direct comparison of the alkaline hydrolysate with an authentic sample of α,α-trehalose by co-TLC in silica and comparing the NMR spectra (Table 1). The position of methoxy group (δ_C 56.5) at C-3 in aglycone part was assigned with the comparison of literature data (Swislocka et al., 2013). The complete assignment of the proton and carbon atoms, their positions and the linkage of the sugar moieties in compound **1** were determined by ^1H–^1H COSY, HMQC and HMBC spectra as given in Fig. 2. Thus, from the physical and spectral studies, the structure of compound **1** was elucidated as 6-*O*-(4-hydroxy-3-methoxy benzoyl)-α,α-trehalose and named as simalin A.

Compound **2** was obtained as colorless gum with negative optical rotation, $[\alpha]_D^{20}$ −47.1°. Its molecular formula was determined as $C_{26}H_{40}O_{17}$ on the basis of a HR-FAB-MS peak of $[M+Na]^+$ at 647.2181 (Calcd. for $C_{26}H_{40}O_{17}Na$, 647.2163). Acid hydrolysis of compound **2** afforded glucose, xylose, and rhamnose as compared in co-TLC in silica gel with authentic samples. The ^1H NMR spectrum of compound **2** showed two equivalent aromatic protons at δ_H 6.42 (2H, s) indicated the presence of a 1,3,4,5-tetrasubstituted phenyl ring. Additionally, two equivalent methoxy groups at δ_H 3.81 (6H, s) along with another methoxy group at δ_H 3.70 (3H, s) revealed the compound contained an aromatic ring with a symmetrical substitution pattern. Moreover the presence of three signals at δ_H 4.93 (1H, d, J = 7.3 Hz), 4.69 (1H, d, J = 1.5 Hz) and 4.63 (1H, d, J = 7.3 Hz) were assigned to three anomaric protons. In agreement with above, the ^{13}C NMR of compound **2** showed signals equivalent to total 26 carbons; including; six aromatic carbons at δ_C 155.8 (C-1), 154.8 (C-3 and C-5), 139.4 (C-4), 97.3 (C-2 and C-6); three methoxy groups at δ_C 56.8 (C-3 and C-5-OCH_3) and 61.2 (C-4-OCH_3) and seventeen carbons assignable to sugar moieties given in Table 2. All signals, except δ_C 101.9, 72.1, 70.2, 70.1, 69.8 and 17.9 (assignable to a rhamnose moiety) were similar with shamiminol (**3**) (Faizi et al., 2011). The downfield shift of –O–CH_2– group in compound **2** suggested the presence of rhamnose group at C-6′ of glucose. The complete assignment of the sugar moieties in the compound **2** were determined by ^1H ^1H COSY, HMQC and HMBC spectra. The HMQC spectrum correlated the anomaric protons δ_H 4.93, 4.69 and 4.63 with anomaric carbon atoms at δ_C at 106.3, 102.0 and 101.9 respectively. The HMBC correlations were observed between methoxy protons at δ_H 3.81 and δ_H 3.70 with aromatic carbons at δ_C 154.8 (C-3 and C-5), and 139.4 (C-4), respectively. The linkage of glucose was supported by the correlation between the anomeric proton (δ_H 4.93) of glucose and C-1 (δ_C 155.8) of phenyl ring in the HMBC spectrum. The linkage of xylose with C-2′ of glucose unit was determined by marked glycosylation shifts, showing an α-effect on C-2′ (resonated at higher frequency) and β-effects on C-1′ and C-3′, which was also supported by the HMBC correlations between anomeric proton of xylose (δ_H 4.63 H-1″) and C-2′ (δ_C 83.7) of glucose. The HMBC spectrum also correlated anomaric proton of rhamnose (H-1‴) at δ_H 4.69 with C-6′ of glucose (δ_C 67.7) (Fig. 2). Thus, from the physical and spectral studies, the structure of compound **2** was elucidated as 3,4,5-trimethoxyphenol-1-*O*[(2→1) xylopyranosyl-(6→1)-α-rhamnopyranosyl]-β-glucopyranoside and named as simalin B.

Table 1
^1H and ^{13}C NMR spectroscopic data of compound **1** and α,α-trehalose.

Position	1*		α,α-Trehalose**	
	δ ^1H (J in Hz)	δ ^{13}C	δ ^1H (J in Hz)	δ ^{13}C
Glc				
1	5.12, d (3.7)	95.1[a]	5.16, d (3.7)	94.6
2	3.53, dd (3.7, 9.8)	72.2	3.59, dd (3.7, 9.5)	72.7
3	3.81–3.84	73.3[b]	3.82–3.85	73.6
4	3.40–3.47	71.6[c]	3.39, dd (9.5, 9.5)	71.3
5	4.15–4.18	74.6[d]	3.81, ddd (2.1, 5.5, 9.5)	74.0
6	4.52, dd (2.1, 11.9)	65.0	3.79–3.80	62.1
	4.40, dd (5.8, 11.9)		3.73, dd (5.5, 12.1)	
Glc				
1′	5.12, d (3.7)	95.2[a]	5.16, d (3.7)	94.6
2′	3.53, dd (3.7, 9.8)	73.2[b]	3.59, dd (3.7, 9.5)	72.7
3′	3.77–3.80	73.9	3.82–3.85	73.6
4′	3.40–3.47	71.9[c]	3.39, dd (9.5, 9.5)	71.3
5′	3.82, ddd (3.9, 5.2, 9.8)	74.8[d]	3.81, ddd (2.1, 5.5, 9.5)	74.0
6′	3.66, dd (5.2, 11.9)	62.6	3.79–3.80	62.1
	3.77–3.84		3.73, dd (5.5, 12.1)	
1″		122.6		
2″	7.75, d (2.1)	113.7		
3″		148.8		
4″		152.9		
5″	6.85, d (7.9)	116.0		
6″	7.58, dd (2.1, 7.9)	125.2		
7″		168.1		
3″-OCH_3	3.90, s	56.5		

[a,b,c,d]Assignments may be reversed in the same column.
* CD_3OD.
** $CD_3OD + D_2O$.

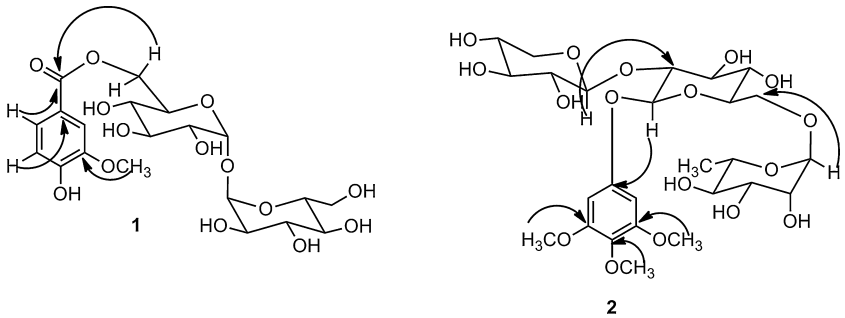

Fig. 2. Selected HMBC correlations (H → C) of compounds **1** and **2**.

The structures of known compounds were identified on the basis of their physical and spectral data by comparison with literature as shamiminol (**3**) (Faizi et al., 2011), (−)-epicatechin-7-*O*-*β*-xylopyranoside (**4**) (Liimatainen et al., 2012), (−)-catechin-7-*O*-*β*-xylopyranoside (**5**), (+)-isolarisiresinol-9′-*O*-*β*-glucopyranoside (**6**) (Otsuka et al., 2000) and (+)-lyoniresinol-9′-*O*-*β*-glucopyranoside (**7**) (Tang et al., 2011).

3. Experimental

3.1. Instruments and chemicals

Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. NMR spectra were measured on a JEOL α-500 spectrometer (^{1}H: 500 MHz and ^{13}C: 125 MHz). Chemical shifts are given in ppm with reference to TMS. Mass spectra were recorded on JEOL JMS 700 MStation mass spectrometer. Column chromatography (CC) was carried out with silica gel 60 (0.040–0.063 mm, Merck), MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Industries Co., Ltd.), Sephadex LH-20 (Amersham Pharmacia Biotech) and Chromatorex ODS (30–50 μm, Fuji Silysia Chemical Co., Ltd.). TLC was performed on precoated silica gel 60 F_{254} plates (0.2 mm, aluminum sheet, Merck). Authentic samples of sugars were obtained from Sigma–Aldrich Chemie, Germany.

3.2. Plant material

The fresh stem bark of *Bombax ceiba* were collected from Sarmoli-6, Pipalkot, Darchula, Nepal in July 2011. The voucher specimen was deposited on Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan (Voucher No.: KUNP20111002-2).

Table 2
^{1}H and ^{13}C NMR spectroscopic data of compound **2** and **3**.

Position	2*		3**	
	δ ^{1}H (*J* in Hz)	δ ^{13}C	δ ^{1}H (*J* in Hz)	δ ^{13}C
1		155.8		154.0
2	6.42, s	97.3	6.36, s	94.9
3		154.8		153.2
4		134.9		132.8
5		154.8		153.2
6	6.42, s	97.3	6.36, s	94.9
3-OCH$_3$	3.81, s	56.8	3.73, s	55.9
4-OCH$_3$	3.70, s	61.2	3.59, s	60.3
5-OCH$_3$	3.81, s	56.8	3.73, s	55.9
Glc				
1′	4.93, d (7.3)	102.0[a]	4.88, d (7.3)	99.1
2′	3.57–3.59	83.7	3.51–3.59	82.3
3′	3.30–3.36	77.4 [d]	3.36–3.39	76.4
4′	3.24–3.28	74.0	3.16–3.18	69.8
5′	3.30–3.36	77.7 [d]	3.43–3.46	77.9
6′	4.02, brd (9.5)	67.7	3.73–3.74	60.9
	3.63, brd (9.5)		3.51–3.59	
Xyl				
1″	4.63, d (7.3)	106.3	4.51, d (7.3)	104.9
2″	4.49–4.50	75.7	3.43–3.46	74.4
3″	3.30–3.36	77.4	3.36–3.39	75.9
4″	3.30–3.39	72.4[b]	3.43–3.46	69.8
5″	3.85, dd (5.2, 11.5)	67.2	3.73–3.74	65.9
	3.24–3.28		3.11–3.14	
Rha				
1‴	4.69, d (1.5)	101.9[a]		
2‴	3.57–3.59	70.1[c]		
3‴	3.57–3.59	70.2[c]		
4‴	3.30–3.39,	72.1[b]		
5‴	3.57–3.59	69.8		
6‴	1.19, d (6.4)	17.9		

[a,b,c,d]Assignments may be reversed in the same column.
* CD_3OD.
** DMSO-d_6.

3.3. Extraction and isolation

The shade dried stem bark (1.1 kg) were extracted successively with 70% MeOH (11 L × 2 times), MeOH (8 L) and water (8 L) at 55 °C (5 h) and room temperature (20 °C, for 22 h) each time. The combined extract was evaporated under reduced pressure to give 118 g extract. The extract (118 g) was suspended in water (500 mL) to give water soluble (112 g) and water insoluble fraction (6 g). The water soluble fraction (112 g) was subjected on MCI gel CHP20P CC and eluted successively with water, 40%, 60%, 80% and 100% MeOH to give twelve fractions (1–12).

Fraction 2 (10.5 g, 40% MeOH eluate) was further subjected to MCI gel CHP20P CC (10–40% MeOH) to give ten subfractions (2-1–2-10). Fraction 2-2 (1.83 g, 10% MeOH eluate) was subjected on Sephadex LH-20 CC (50% MeOH) to give ten subfractions (2-2-1–2-2-10). Fraction 2-2-3 (174 mg) was applied on ODS CC (15% MeOH) to give five subfractions (2-2-3-1–2-2-3-5). Fraction 2-2-3-4 (17 mg) was applied on silica gel CC ($CHCl_3$:MeOH:H_2O = 8:2:0.1) to afford compound **1** (11 mg). Fraction 2-2-5 (97 mg) was applied on ODS CC (10% MeOH) to afford compound **4** (48 mg). Fraction 2-2-6 (225 mg) was applied on ODS CC (10% MeOH) to afford compound **5** (13 mg). Fraction 2-3 was (546 mg, 10% MeOH eluate) was subjected on Sephadex LH-20 CC (50% MeOH) to give six subfractions (2-3-1–2-3-6). Fraction 2-3-1 (134 mg) was applied on ODS CC (15% MeOH) to give three fractions (2-3-1-1–2-3-1-3). Fraction 2-3-1-2 afforded compound **3** (19 mg). Fraction 2-3-1-3 (25 mg) was applied on silica gel CC ($CHCl_3$:MeOH:H_2O = 8:2:0.1) to afford compound **2** (9 mg). Similarly, fraction 5 (452 mg, 40% MeOH eluate) and 6 (265 mg, 40% MeOH eluate) were mixed together and applied on ODS CC (30% MeOH) to give ten subfractions (5-1–5-10). Fraction 5-4 (190 mg, 30% MeOH eluate) was applied on Sephadex LH-20 CC (MeOH) followed by ODS CC (25% MeOH) to afford compound **7** (28 mg). Subfraction 5–6 (62.1 mg, 30% MeOH eluate) was applied on MCI gel CHP20P CC (20–35% MeOH) followed by silica gel CC ($CHCl_3$:MeOH:H_2O = 8:2:0.1) to afford compound **6** (16 mg).

3.4. Simalin A (*1*)

Colorless gum; $[\alpha]_D^{20}$ +80.4° (c 0.61, MeOH); HR-FAB-MS (positive mode) $[M+Na]^+$ at 515.1380 (Calcd. for $C_{20}H_{28}O_{14}$ Na, 515.1377); ^{13}C and ^1H NMR data are given in Table 1.

3.5. Simalin B (*2*)

Colorless gum; $[\alpha]_D^{20}$ −47.1° (c 0.81, MeOH); HR-FAB-MS (positive mode) peak of $[M+Na]^+$ at 647.2181 (Calcd. for $C_{26}H_{40}O_{17}Na$, 647.2163); ^{13}C and ^1H NMR data are given in Table 2.

3.6. Known compounds

Shamiminol (**3**): colorless gum, $[\alpha]_D^{20}$ −25.6° (c 0.25, pyridine); (−)-epicatechin-7-*O*-*β*-xylopyranoside (**4**): pale yellow amorphous powder, $[\alpha]_D^{20}$ −48.5° (c 0.91, MeOH); (−)-catechin-7-*O*-*β*-xylopyranoside (**5**): pale yellow amorphous powder, $[\alpha]_D^{20}$ −56.6° (c 0.77, MeOH); (+)-isolarisiresino-9′-*O*-*β*-glucopyranoside (**6**): white amorphous powder, $[\alpha]_D^{20}$ +39.7° (c 0.29, MeOH); (+)-lyoniresinol-9′-*O*-*β*-glucopyranoside (**7**): white amorphous powder, $[\alpha]_D^{20}$ +29.7° (c 1.12, MeOH).

4. Hydrolysis of compound 1 and 2

Compound **1** (0.5 mg) was dissolved in 3% KOH–MeOH (1 ml) and kept at room temperature for 2 h. The mixture was neutralized with 1 M HCl. Compound **2** (1 mg) in 2 M HCl (0.2 mL) was refluxed at 70 °C for 3 h. Reaction mixtures were separately subjected to silica gel TLC, together with the standard samples, using $CHCl_3$–MeOH–H_2O (6:4:1, v/v/v) and n-BuOH–AcOEt–H_2O (5:1:4, v/v/v, upper phase) as the developing solvents and using 10% aqueous H_2SO_4 as the detection reagent. *α*,*α*-Trehalose was detected from the compound **1** and glucose, xylose and rhamnose were detected from the compound **2**.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2013.09.005.

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